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Short Communication

Levetiracetam in submaximal subcutaneous pentylentetrazol-induced seizures in rats

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ABSTRACT

Despite anticonvulsant efficacy in animal models of generalized epilepsy, levetiracetam was not effective in the maximal subcutaneous PTZ model in mice and rats.

Aim of this study was to assess the efficacy of levetiracetam (LEV) against submaximal, s.c. MET test (PTZ at the dose of 70 mg/kg) acute seizures in Wistar rats, in comparison to valproic acid (VPA).

Thirty male Wistar rats (P42) were divided in three drug-treatment groups (10 rats in each group) as follows: valproic acid, levetiracetam, and controls. All animals were tested for seizure threshold at age P50. VPA (110 mg/kg) and LEV (108 mg/kg) were freshly dissolved in saline and injected i.p. in 2–3 ml/kg, 15 and 30 min, respectively, before pentylenetetrazol (PTZ) injection at the dose of 70 mg/kg.

The average latency of the seizure type 3 (generalized clonic seizure with loss of righting reflexes) significantly differed between controls and the drug-treated animal groups ($p \leq 0.02$). The average duration of the seizure type 2 (threshold seizure) was significantly longer in both groups compared to controls (<0.02).

In conclusion, LEV plays a role against seizures triggered by subcutaneous PTZ injection given at submaximal doses in rats, as demonstrated by a significant increase in duration of the seizure type 2 (threshold seizure).

1. Introduction

Levetiracetam (LEV) is a new generation drug which was effective for the treatment of focal seizures with or without secondary generalization, as well as tonic-clonic and myoclonic generalized seizures.[1–4] Recent report confirmed LEV to be also effective in controlling absence seizures,[5] and placebo-controlled trials with this drug are ongoing.

In preclinical studies, levetiracetam has been shown active in animal models that are believed to represent generalized seizures. The expression of seizure activity was prevented in both audiogenic susceptible mice[6] and rats.[7] A marked suppression of the mean duration of spike-and-wave discharges was also obtained by levetiracetam in rats from the Genetic Absence Epilepsy Rats from Strasbourg (GAERS) strain even at the lowest tested dose. A similar suppression of pentylenetetrazol (PTZ)-induced spike-and-wave discharges was also observed with an i.p. dose of 17 mg/kg[6].

Despite such a clinical evidence in humans and animal models of generalized epilepsy, LEV was not effective at doses up to 500 mg/kg in the experimental animal model of maximal s.c. PTZ at the dose of 90 mg/kg, in mice and rats.[8] This model has been hypothesized to correlate with the efficacy of an anticonvulsant drug against myoclonic and clonic seizures in humans.[9] Yet, the type and severity of the generalized seizures induced in this model are related to the dose and route of PTZ injection.[10,11]

It is well accepted that maximal pentylentetrazole test (MMT) and maximal electroshock test (MES) are considered "suprathreshold" tests, while "threshold tests" include the PTZ infusion test and the threshold electroconvulsive test (ECS). Among these, the subcutaneous pentylentetrazol (scMET) test[12] may be included, since a lower dose of PTZ (70 mg/kg) is given instead of 90 mg/kg, as in the MMT test.

While suprathreshold tests are best suited to study maximal seizures in rodents, consisting of tonic forelimb and hindlimb extension, and are used in drug development to model tonic-clonic

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seizures in humans, threshold tests can better assess minimal (threshold) seizures consisting of face and forelimb clonic jerks, and are used in drug development to model myoclonic seizures in humans.[9]

Such an experimental model may indeed contribute to better disclose the anticonvulsant properties of a new drug, i.e. by comparing them with those of a well established one.

In order to further assess the actual efficacy of LEV in such a seizure model, specific for generalized seizures of clonic and myoclonic type, we evaluated LEV efficacy against s.c. MET test (PTZ at the dose of 70 mg/kg) acute seizures in Wistar rats.

2. Materials and methods

2.1. Animals and diet

Thirty male Wistar rats (Harlan Italy, Milan, Italy) were housed in groups of two in polycarbonate cages at a temperature of 25 °C, on an alternating 12-h light/12-h dark cycle with lights on at 06:00 h.

Animals arrived at 42 days of age (P42) and were fed rodent chow (F1515 Rodent diet, AIN-76A, 1/2 pellets by Bio-Serv, Frenchtown, NJ, USA) and provided water ad libitum for 8 days before initiation of the experiment.

All animals were maintained within conditions specified in approved Institutional Animal Care and use Committee protocols. The experiment protocol was previously approved by the local Ethic Committee.

2.2. Seizure threshold

After maintenance on a free chow diet, all animals were divided in three drug-treatment groups (10 rats in each group) as follows: valproic acid, levetiracetam, and controls.

Valproic acid (VPA), supplied by Sanofi-Aventis as purified product, was dissolved in 0.9% NaCl, sterile filtered (0.2 μm, Coster), and injected intraperitoneally (i.p.) at the dose of 110 mg/kg. This VPA dosage was reported to be the ED50 in Wistar rats by Löscher et al.[9].

Levetiracetam (LEV), supplied by UCB-Belgium as purified product, was dissolved in 0.9% NaCl sterile filtered (0.2 μmol, Coster) and injected intraperitoneally (i.p.) at the dose of 108 mg/kg. This dose, effective in the e.v. PTZ model 13), was chosen to be assessed in a submaximal s.c. PTZ seizure model.

Controls were given an equivalent volume of saline (i.p.). All animals were tested for seizure threshold at age P50.

Pentylenetetrazol (PTZ) (Sigma Chemical Co.) was dissolved in bacteriostatic saline (Abbott) to a concentration of 10 mg/ml and injected at the dose of 70 mg/kg subcutaneously into a loose fold of skin on the back of the neck of the animals. The PTZ seizure threshold test was administered according to a modification of the procedure by Krall et al.[14] The dose of 70 mg/kg was considered intermediate between the maximal dose (90 mg/kg) and 50 mg/kg, which was determined in a separate experiment as the minimal dose required to induce generalized seizures in 100% of our rats within approximately 30 minutes after PTZ injection.

Valproic acid (110 mg/kg) and levetiracetam (108 mg/kg) were freshly dissolved in saline and injected i.p. in 2–3 ml/kg, 15 and 30 min (time to maximum serum concentration) before PTZ injection, respectively.[9,15,16]

Soon after PTZ administration, each rat was video-taped and monitored for 1 h, and the different seizure types (in order of appearance) were rated as follows: 0, no seizures; 1, generalized myoclonic twitches; 2, generalized clonic seizure without loss of righting reflexes ("threshold seizure"); 3, generalized clonic seizure with loss of righting reflexes; 4, loss of righting reflexes with forelimb tonus; 5, loss of righting reflexes with hindlimb tonus.[9]

Since PTZ was given at the dose of 70 mg/kg, that is less than the CD97 (90 mg/kg by Löscher et al.[9]), rats with no seizures (seizure type 0) were excluded from the study. Following the same reason, the video monitoring of each rat was prolonged for at least 1 h after PTZ injection.

All animals were seizure naïve when tested and each was subjected to seizure testing only once. Seizures were always induced between 13:00 and 17:00 to minimize possible complicating effects of circadian rhythms[17].

2.3. Statistical analysis

One-way analysis of variance was performed to evaluate the relationship between groups of treatment and Dunnett *t*-test was used to compare all other groups against control. A p value below 0.05 was considered significant.

3. Results

All rats developed seizures leading to exitus. While there was no significant difference among the three groups as regards the start of the seizures type 1 and 2, the average latency of the seizure type 3 significantly differed between controls and the other two groups ($p \leq 0.02$; Dunnett *t*-test, two-sided). Furthermore, the average latency (in seconds) was essentially overlapping both in VPA and LEV group (1159.43 ± 928.0 and 1477.43 ± 615.0) (Fig. 1).

Fig. 2 shows the mean duration ± SD of the seizures type 1 and 2 (seizure threshold) in each group of animals. While duration of the seizure type 1 was only slightly increased in the VPA group, the seizure type 2 lasted significantly longer in both groups, compared to controls ($p \leq 0.02$).

4. Discussion

In the present study, levetiracetam has increased, to the same extent of valproic acid, the seizure threshold in rats that were injected with s.c. PTZ at the dose of 70 mg/kg. In previous studies, levetiracetam resulted ineffective in the experimental model of s.c.

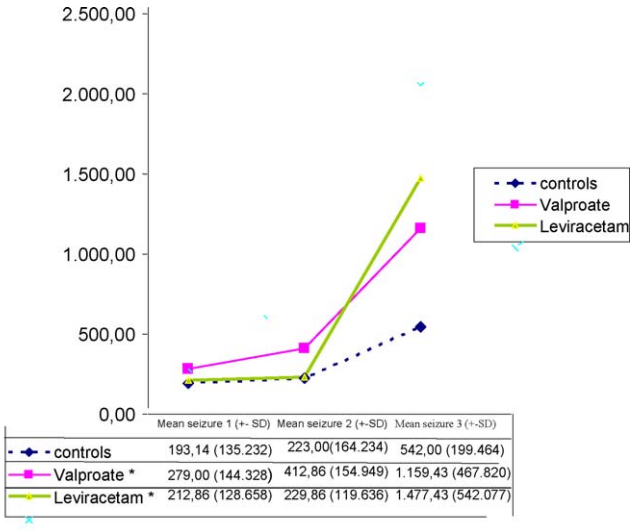
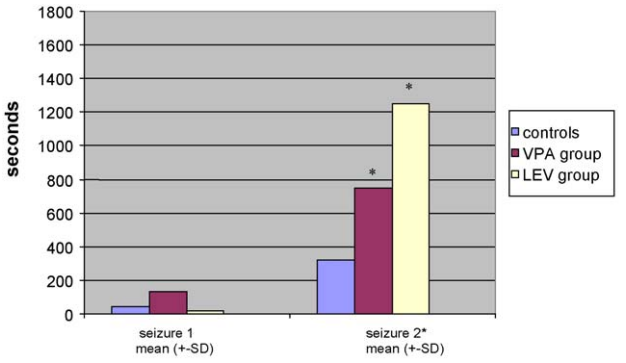


Fig. 1. Latency (seconds) of different seizure types in the three groups of rats. *$p \leq 0.02$ (Dunnett *t*-test, two-sided (seizure latency in controls vs. the other groups).



Controls	45.3 (34.9)	326.3 (59.1)
Valproic acid	135.0 (106.2)	805.2 (476.9) *
Levetiracetam	15.6 (8.2)	1119.8 (593.0) *

*, p= ≤ 0.02 (Dunnett t-test, two-sided; seizure type 2 in controls vs the other groups)

Fig. 2. Mean duration of seizure 1 and 2 after s.c. PTZ 70 mg/kg in Wistar rats. $^{*}p \leq 0.02$ (Dunnett *t*-test, two-sided; seizure type 2 in controls vs. the other groups).

PTZ at the dose of 90 mg/kg,[8,13,18] that is the dose inducing the first generalized clonic seizure with loss of righting reflexes in 97% of the rats (CD97).[9]

A lower dose of PTZ (70 mg/kg) probably allowed the anticonvulsant action of levetiracetam to emerge also in this experimental model of acute induced generalized seizures. Similarly, a lower dose of s.c. PTZ (50 mg/kg) disclosed seizure protection against seizures in Wistar rats fed the ketogenic diet.[19]

This data explains why anticonvulsant drugs, as vigabatrin and progabide, were also ineffective in the s.c. PTZ model (supra-threshold test), while they were effective in the i.v. PTZ seizure model.

The generalized clonic seizure type 2, specifically correlated with the seizure threshold by Swinyard[11] in this experimental model, lasted significantly longer in both the drug-treated animal groups than controls. Transition to the seizure type 3 (initial loss of the righting reflexes with generalized clonic seizures) was thus delayed in the same groups of rats.

The latency of the seizure type 2 was, conversely, not influenced by any of the tested drugs. This result is in keeping with the fact that seizure type 2 (generalized clonic) is the most suitable to assess the anticonvulsant potency in the s.c. PTZ model, while seizure type 1 (myoclonic twitches) is best indicated for studying seizure threshold in the i.v. PTZ model.[9,11]

The potency of an anticonvulsant drug against PTZ-induced seizures type 2 seems, further, to predict anticonvulsant activity against myoclonic and/or clonic seizures in humans more than absence seizures. Löscher et al.,[9] in fact, suggest that drugs like phenobarbital and primidone, neither of which exerting therapeutic effects against absence seizures in humans, are effective in the PTZ seizure model, similarly to valproate and ethosuximide.

Data coming from this experiment, based indeed on submaximal s.c. PTZ doses, show an anticonvulsant action of levetiracetam closely paralleling that of valproic acid. They are therefore in keeping with the clinical efficacy of valproic acid[20] and levetiracetam against such a kind of epileptic seizures in humans.[5]

Nonetheless, submaximal doses of PTZ may be hypothesized to involve pharmacodinamic interactions other than those implicated in the supramaximal s.c. PTZ model, for which the intrinsic mechanisms are not well defined.

Sequencing of the different seizure types is however overlapping in both seizure models, somewhat meaning the involvement of the same brain networks.

Video monitoring lasting at least 1 h was necessary because of the potential increase in latency of the different seizure types due to the lower PTZ dose. Potential inter-animal variability of PTZ absorption from the subcutaneous compartment was minimized by a sufficient number of rats in each group with a homogeneous strain, body weight and age.

Seizures could, therefore, develop within or far beyond 30 min after PTZ was injected subcutaneously.

Both drugs were tested at the time of maximum effect as determined in MES experiments,[9,18] because it is unlikely that the time of peak effect is different in different seizure models.[9]

In conclusion, this trial shows levetiracetam to play a role against seizures triggered by subcutaneous PTZ injection given at submaximal doses in rats, as demonstrated by a significant increase in duration of the "threshold" seizure type 2 and latency of the seizure type 3. These data are in keeping with the clinical efficacy of LEV against generalized tonic-clonic and myoclonic seizures in humans.

Further, this study confirmed the advantages of the submaximal s.c. PTZ test, compared to the PTZ tail vein infusion model, consisting of its simplicity and possibility of fast and simultaneous assessment of several seizure parameters.[19] While the PTZ infusion model can accurately evaluate the threshold of seizure onset, the s.c. MET model allows to assess the spreading of the different seizure types according to their origin and diffusion in the different brain networks such as forebrain (clonic seizures) or brain stem (tonic seizures).

Conflict of interest

None of the authors has any conflict of interest to disclose.

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We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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